Metal-Containing Oligonucleotides: Solid-Phase Synthesis and Luminescence Properties

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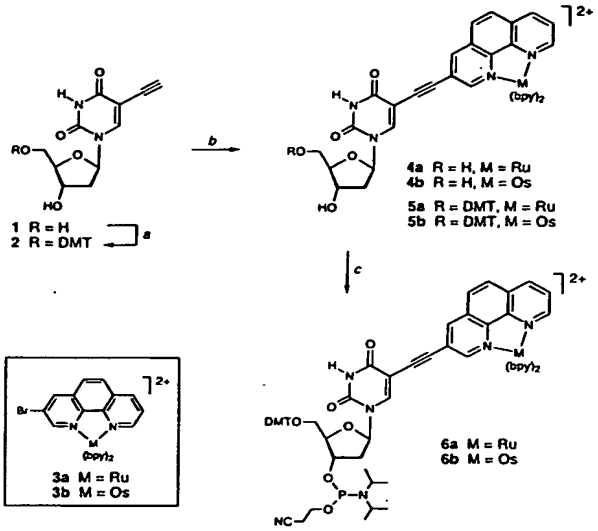
The incorporation of photo- and redox-active transition metal ions into oligonucleotides is a key design target for the study of energy and electron-transfer processes through DNA,[1] as well as the development of DNA hybridization probes and sensors.[2] Metal-containing oligonucleotides have been predominantly constructed via two major pathways: (a) the synthesis of a chelator-containing oligonucleotide followed by metal complexation[3] and (b) the synthesis of an end-functionalized oligonucleotide to which a metal complex can be conjugated.[1,4] These approaches are restricted primarily to modifications at the oligonucleotides' termini and/or require the exposure of oligonucleotides to reactive metal precursors.[2-5] A direct method for the site-specific incorporation of metal complexes during solid-phase oligonucleotide synthesis has never been reported.

We now disclose a general methodology for the incorporation of polypyridine metal complexes into oligonucleotides using automated DNA synthesizers. We report the synthesis of novel Ru^{II}- and Os^{II}-containing nucleosides and their phosphoramidite derivatives. These building blocks are sequence-specifically incorporated into oligonucleotides in high yields using standard solid-phase phosphoramidite chemistry. The uniquely modified oligonucleotides form stable DNA duplexes and are useful probes for the study of energy-transfer processes in nucleic acids.

We have previously reported that functionalized tris-chelate complexes are excellent substrates for the powerful palladium-mediated cross-coupling methodologies.[6] This approach provides a convenient entry into metal-containing nucleosides and is key to the successful preparation of the modified nucleosides and their phosphoramidites. Thus, palladium-catalyzed cross-coupling reactions between 5-ethynyldeoxyuridine[7] (**1**) and $[(bpy)_2Ru(3\text{-bromo-}1,10\text{-phenanthroline})]^{2+}(PF_6^-)_2$ (**3a**) or $[(bpy)_2Os(3\text{-bromo-}1,10\text{-phenanthroline})]^{2+}(PF_6^-)_2$ (bpy = bipyridyl) (**3b**) afford nucleosides **4a** and **4b**, respectively (Scheme 1).[8,9] The mild conditions of this reaction allow us to apply it for the modification of 4,4′-dimethoxytrityl-protected nucleosides. Thus, **1** is first treated with 4,4′-dimethoxytrityl (DMT) chloride in the presence of 4-(dimethylamino)pyridine (DMAP) to provide the DMT-protected nucleoside **2**, which is then cross-coupled to **3a** or **3b** to afford the protected metal-containing nucleosides **5a** and **5b**, respectively (Scheme 1). Phosphitylation of the protected nucleosides **5a** and **5b** using (2-cyanoethoxy)bis(diisopropylamino)-phosphine in the presence of (1*H*)-tetrazole provides the corresponding metal-modified phosphoramidites **6a** and **6b**.[10]

Target 20-mer oligonucleotides incorporating one or two metal-modified 2′-deoxyuridine bases at various positions were synthesized on a 0.2 μmol scale using an automated DNA synthesizer (Figure 1). When coupling times for the phosphoramidites **6a** and **6b** in 0.5 M (1*H*)-tetrazole were extended to 5 min, reaction efficiencies were greater than 90%.[11] Removal of the finished 20-mers from the solid support using concentrated ammonium hydroxide was followed by incubation at 55 °C for 8 h to afford

Scheme 1. Synthesis of Phosphoramidites **6a** and **6b**[a,b]



[a] Reagents: (a) 4,4′-dimethoxytrityl chloride (DMT-Cl), DMAP, pyridine, Et_3N, 92% yield; (b) **3a** or **3b**, $(Ph_3P)_2PdCl_2$, CuI, DMF, Et_3N, sonication, 84% yield; (c) $(iPr_2N)_2POCH_2CH_2CN$, (1*H*)-tetrazole, CH_3CN; 70−85% yield. [b] All metal-modified nucleosides were isolated as their PF_6^- salts.[8]

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(11) To control the amount of reagents and reaction time, the coupling of the modified bases was performed manually (ref 8). The decreased coupling efficiencies relative to standard phosphoramidites are likely due to the steric bulk of the appended metal complex and have been reported with other bulky phosphoramidites. See: Kobertz, W. R.; Essigmann, J. M. *J. Am. Chem. Soc.* **1997**, *119*, 5960−5961.

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7	5'	TCG	GCG	CGA	ATT	CGC	GTG	CC	3'
8	5'	TCG	GCG	CGA	A**Ru**T	CGC	GTG	CC	3'
9	5'	TCG	GCG	CGA	A**Os**T	CGC	GTG	CC	3'
10	5'	**Ru**CG	GCG	CGA	ATT	CGC	GTG	CC	3'
11	5'	TCG	GCG	CGA	A**Os**T	CGC	G**Ru**G	CC	3'
12	3'	AGC	CGC	GCT	TAA	GCG	CAC	GG	5'
13	3'	AGC	CGC	GC**Os**	TAA	GCG	CAC	GG	5'
14	3'	AGC	CGC	GCT	TTA	GCG	CAC	GG	5'

Figure 1. Sequences of oligonucleotides synthesized. The Ru^{II}- and Os^{II}-containing deoxyuridine nucleosides (4a and 4b, respectively) are shown in bold.

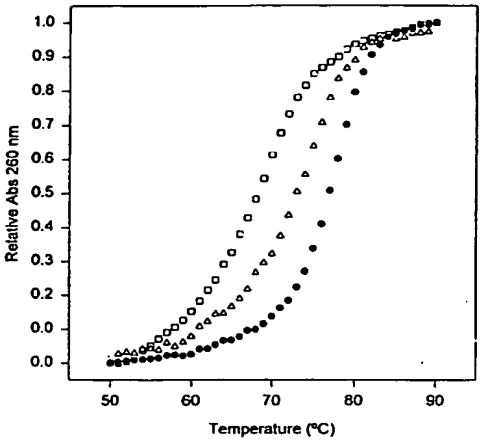

Figure 2. Thermal denaturation curves for control duplex **7·12** (●), Os^{II}-containing duplex **9·12** (△), and a single-mismatch duplex **7·14** (□) determined in 0.01 M sodium phosphate buffer pH 7, 0.1 M NaCl.[8]

the deprotected oligomers **8**−**11** and **13** that were purified by gel electrophoresis.[8] Analytical denaturing polyacrylamide gel electrophoresis confirmed the purity of the modified oligonucleotides, and enzymatic digestion followed by HPLC analysis verified the presence of the intact metal-containing nucleosides.[8]

The presence of the a metal-containing nucleoside has a relatively small effect on duplex stability as determined by thermal denaturation curves (Figure 2). The melting temperature (T_m) of the unmodified duplex derived from oligonucleotide **7** and its complementary sequence **12** is 78 °C. When the metal-containing nucleoside is located at the 5′-end, as in duplex **10·12**, the T_m is essentially the same. Duplexes **8·12** and **9·12**, in which the metal-containing nucleoside is in the middle of the duplex, are slightly less stable with a T_m at 75 °C. Yet, this destabilization is far smaller than the effect of a single mismatch on duplex stability as demonstrated for duplex **7·14** containing a T−T "pair" at the same position (T_m = 69 °C, Figure 2).

Steady-state emission profiles of iso-absorptive oligonucleotide solutions in degassed phosphate buffer are shown in Figure 3. The Ru^{II}-containing duplex **8·12** shows a typical metal-centered emission at 630 nm upon excitation of the visible metal-to-ligand charge-transfer (MLCT) band at 456 nm. Upon hybridization of the Ru^{II}-containing oligonucleotide **8** to a complementary Os^{II}-containing oligonucleotide **13**, a substantial drop in the emission intensity (ca. 70−85%) is observed. This suggests an "intra-duplex" quenching of the excited Ru^{II} center by the proximal Os^{II}

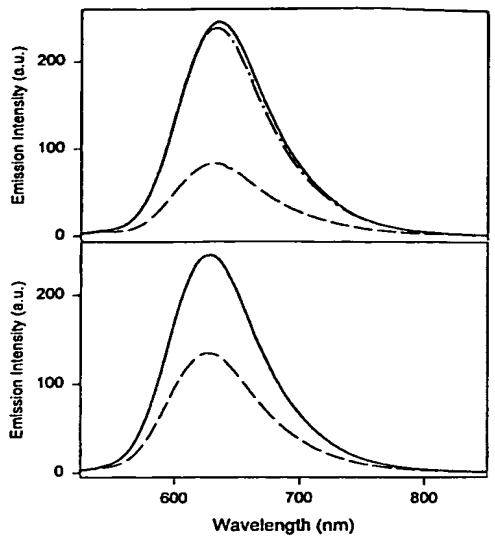

Figure 3. Steady-state emission spectra of modified oligonucleotides in degassed 0.01 M sodium phosphate buffer pH 7.0, 0.1 M NaCl. Top: duplex **8·12** (solid line), a 1:1 mixture of **8** and **9** (-·-), and a duplex containing proximal Ru and Os **8·13** (dashed line). Bottom: duplex **10·12** (solid line) and duplex **10·13** (dashed line).

center.[9,12] Intermolecular quenching can be excluded since a 1:1 mixture of the noncomplementary oligonucleotides **8** and **9** shows essentially the same emission intensity as duplex **8·12** (Figure 3a, top). This behavior is distance-dependent as demonstrated by comparing duplex **8·13** to duplex **10·13**. In this case, where the Os^{II} center is more remote, only 40% quenching of the Ru^{II}-based emission is observed (Figure 3b, bottom). To the best of our knowledge, this is the first example of energy-transfer processes in DNA oligonucleotides that are sequence-specifically modified with polypyridine metal complexes.

The data presented here establish a novel and powerful approach for the site-specific incorporation of polypyridine−metal complexes into synthetic oligonucleotides using automated phosphoramidite chemistry. The versatile phosphoramidite synthesis and the compatibility with existing automated DNA synthesizers provides enormous flexibility for rapid construction of oligonucleotide−metal conjugates. The presence of photo- and redox-active metal centers in these oligonucleotides makes them extremely important probes for the study of photophysical processes in nucleic acids.

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Supporting Information Available: Synthetic procedures and analytical data for all new derivatives as well as procedures and data for oligonucleotide synthesis, purification, digestion, melting, and fluorescence studies (13 pages). See any current masthead page for ordering information and Web access instructions.

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